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'BROWN OAK' AND ITS ORIGIN

[Annals of Botany, Vol. XXIX, No. CXV, July, 1915.]

'Brown Oak' and its Origin.

BY

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THE ordinary heart-wood of certain individual trees of *Quercus Robur* in Great Britain is sometimes partially replaced by a firm, richer-toned, often reddish, brown wood known as 'brown oak' or 'red oak'. Such wood varies in tint from dull brown to rusty brown, or even to rust-coloured. Sometimes it is uniformly coloured, but at other times longitudinal bands of more or less normal-coloured heart-wood alternate with deep or bright brown streaks, which may include nearly black patches. This latter type of 'brown oak' is the so-called 'tortoiseshell' variety.

Trees possessing 'brown oak', so far as is known, show no external differences from normal individuals. This is not surprising as the heart-wood is the sole part affected, the remaining tissues being normal in structure and apparently in dimensions. In particular the sap-wood seems to be of ordinary size, and the sole evidence suggesting a possible precocious conversion of sap-wood into heart-wood is limited to the occasional presence of scanty starch-grains in a few cells of medullary rays in the heart-wood. Some 'brown-oak' trees are stag-headed. This condition induced by the desiccation of the branches is known to be caused by a number of influences that either check the supply of water to the foliage, or induce excessive transpiration. The evidence provided later in this paper proves that there is no reason to believe that the stag-headed condition of 'brown-oak' trees is directly induced by the influence responsible for the production of the brown heart-wood.

General Distribution of 'Brown' Wood.[1]

In the trunk the brown wood most frequently occurs at the base, extends upwards to a variable height, and usually tapers in such a manner that its topmost portion apparently coincides with the inmost heart-wood. Not infrequently, however, in its upward taper the 'brown heart' becomes gradually confined to one side of the trunk. A special example of this was

[1] For many of the facts concerning the general distribution of 'brown oak' in the individual tree I am indebted to Messrs. Alexander Howard and Stuart Oliver, the former of whom informs me that he has seen 'brown oak' in a specimen of *Quercus rubra.*

afforded at Radlett by a tree, whose brown wood at the base of the trunk extended (apparently) completely across the heart-wood, then tapered very sharply in an upward direction, becoming at the same time confined to one side of the trunk, and continuing thus upwards, gradually tapering to extinction at an approximate height of 15 feet.

In connexion with this partially unilateral arrangement may be mentioned the interesting case of an oak-tree that grew near a stream. The bole, only 18 inches in height, gave way to two erect stems, each of which was about 18 inches in thickness over a length of 12–15 feet. The stumpy bole showed 'brown oak' on one side only, and the erect leader topping that side also was characterized by 'brown' wood, whereas the other leader springing from the half of the bole possessing normal heart-wood contained no 'brown oak'.

This case leads to the consideration of those pollard oaks in which the main trunk includes 'brown oak' extending up to the region of insertion of the branches, which form the multiple leaders of the crown. In the majority of cases 'brown oak' is contained by only one or, perhaps, two of the several leaders, and these are inserted above that section of the main trunk which is characterized by the strongest and richest colour in its brown heart; in such cases the remaining leaders show heart-wood normal in colour. Yet occasionally all the leaders (five in one case) include rich 'brown oak'.

'Brown oak' extending *upwards* in the trunk is usually arrested by extensive knots, and, in any case, a large knot acts as an obstacle. Consequently, in such cases, large boughs springing from the *side* of the trunk are apt to be devoid of 'brown oak'. The reason for this will be at least partly evident, when the cause of the browning of the heart-wood is explained later in this paper.

When 'brown oak' occurs at the base of the trunk it also extends downwards into the main root, where it tapers in the same manner as in the trunk.

Apparently 'brown oak' may occur in upper parts, including the crown, of a tree whose more basal parts are devoid of it. Very significant in relation to the cause is Mr. A. Howard's statement as follows: 'it appears probable that in a few cases the brown wood may start from a large knot below the crown, and extend somewhat downwards'. Mr. Stuart Oliver mentions one case in which the brown wood extended completely across the heart at the base of the bole, and upwards for some distance, then disappeared, only to recur at a height of 35 feet where the bole forked.

Age of trees possessing 'brown oak'. While on the one hand very old oak-trees contain 'brown oak', the minimum age at which the tree can acquire it is apparently determined by that at which the heart-wood

normally appears. Trees with a maximum diameter of trunk of 12 inches frequently contain such wood, which is to be seen in individuals whose maximum estimated age was twenty years.

'Brown-oak' trees in relation to site. Of oak-trees growing close together some may be normal, and others may be 'brown-oak' trees. For instance, at Farming Woods Park, of seven oak-trees close together, only one had 'brown oak'; whereas in a small wood at Stanmore in Middlesex the majority of oak-trees had thoroughly brown heart-wood, others had their heart-wood brown to a slight extent, and yet others were quite normal.

Previous Hypotheses as to the Cause of the Production of 'Brown Oak'.

Up to the present the cause of the production has been unknown, but three hypotheses have been put forward assuming that it is:

(i) Due directly to some *chemical* body locally abundant in the soil.

(ii) Due to *incipient decay.*

(iii) A mere *sport.*

(1) The *chemical* hypothesis not only has no evidence in its favour, but is rendered improbable by the facts concerning the occurrence side by side on the same site of normal and 'brown-oak' trees, and concerning the distribution of 'brown oak' in the individual tree, and especially those cases of its unilateral or discontinuous distribution in the trunk. The chemical constituent frequently suggested is iron. Published analyses show that iron may be feeble or abnormally abundant in the wood, and yet this may be normal. An analysis conducted in the Chemical Laboratory of the Imperial College also shows that the amount of iron in 'brown oak' does not necessarily exceed that in normal heart-wood. The following was the result of the analysis:

	Ash.	*Iron (as Fe).*
Normal heart-wood	0·17 %	0·01 %
Brown "	0·5 %	0·01 %

(2) *Incipient decay* has often been suggested as the cause of the appearance of 'brown oak'. Such a view seems to imply that the browning of the heart-wood is an early stage of a process which culminates in the rotting and disintegration of the affected wood. Calculated to arouse such a suspicion are the following facts: Freshly felled 'brown-oak' trees often show the brown heart in a condition of decay, or the trunk partly hollow. Occasionally, at the base of such trees, the heart-wood up to a height of from 3 to 6 feet is converted into a mass of wood rendered white by decay, while above this is firm and hard typical 'brown oak'. Finally, an interesting and significant fact is mentioned by Mr. Alexander Howard, namely

that often it is found that 'brown-oak' trees have lost their tap-root, whose functions are carried on by massive lateral roots.

Not one of these facts indicates definitely that the influence causing the rotting of the wood is identical with that causing the heart to become 'brown oak'. The facts merely indicate that in the case of these trees with rotten heart-wood, there are facilities for the entrance, through wounds, of fungi capable of attacking heart-wood of the oak, and that in certain cases those facilities are especially marked near the base of the trunk and roots. In the sequel it will be possible to discuss if the cause of browning and rotting of the wood is the same.

Deepening in the colour of wood, such as is undergone by alder, or by the heart-wood of larch, pine, and other trees, on exposure to air and light is assumed to be of a purely chemical nature, and at least often is due to oxidation (which is also largely responsible for the change of tint in sap-wood that is being converted into heart-wood). But, with the exception of the still unexplained cases of the 'red heart' of beech and of French oak (*Chêne rouge*), considerable deviations from the normal colour of the heart-wood are invariably due to the action of fungi. Yet no observer has recorded the presence of a fungus in firm 'brown oak' that shows no macroscopic symptoms of decay. And against the suggestion that a fungus or bacterium is responsible for the replacement of normal heart-wood by 'brown oak', is the fact that good 'brown oak' is to the timber-merchant or builder strong, hard, sound wood, responding to tools as does normal oak timber. It is true that species of *Ceratostomella*—for instance *C. Pini*—living in the sap-wood weakens the wood little or not at all, as its attack on wood-substance is slight; but in this case the fungus feeds on the carbohydrate and proteid food of the medullary rays and wood-parenchyma: such food is not generally available to any fungus living exclusively in heart-wood.

Therefore there was no adequate reason for believing that 'brown oak' owes its origin indirectly to a fungus or other foreign organism, or is the first step in a process of decay.

(3) The third hypothesis, namely that 'brown-oak' trees are mere sports, can be dealt with only after the evidence supplied in the sequel. Yet it may be pointed out that on such a hypothesis the occasional uneven distribution of 'brown oak' on the same site or in one and the same tree would find analogies in connexion with some tropical trees, including ebonies (e.g. *Diospyros Kurzii* and *D. Melanoxylon*), whose true dark heart-wood shows similar varied development.

TRUE CAUSE OF PRODUCTION OF 'BROWN OAK'.

As it seemed possible that the reason of our ignorance as to the cause of the production of 'brown oak' lay in the lack of any microscopical observations at the critical stage of its origin, I secured specimens of wood of freshly

felled 'brown-oak' trees. These came from different districts and were kindly supplied by Messrs. William Oliver & Sons, Messrs. Bradley, and Dr. Borthwick of Edinburgh. Two of the three specimens clearly showed wood in the process of conversion into 'brown oak'. Those specimens proved that 'brown oak' is confined to the heart-wood, and is not produced by a change in the sap-wood, but *first passes through the stage of being normal heart-wood.* The first visible macroscopic change is a superposition of a yellow coloration on the normal colour of the heart-wood; this is succeeded by a deepening of tint until the full-coloured brown stage is attained. With the naked eye in one specimen (Oliver's) it was possible to see that the browning took place in the heart-wood along longitudinal strands, causing the wood to be brown-striped in longitudinal section, and dotted with brown islands in transverse section.

Proof of the presence of a living fungus in the heart-wood during conversion into 'brown oak'.

Pieces of the freshly felled wood were placed under running tap-water for 6–12 hours, and then placed above water in sterilized potato-dishes. From the exposed transverse section pure white fungal hyphae grew forth, almost exclusively from regions of the heart-wood, where conversion into 'brown oak' was in progress. No hyphae came from the sap-wood. The Edinburgh specimen showed a solid core of 'brown oak', and practically no normal heart-wood; the hyphae thus emerged from the periphery of the 'brown oak' in close vicinity to the sap-wood. But Oliver's specimen showed in succession within the sap-wood a layer of normal heart-wood, one of brown-striped heart-wood, and a solid core of 'brown oak'. Accordingly the hyphae emerged solely from the periphery of the solid core, and also from the brown strips and peripheries of these in the striped wood.

The whiteness of the colour of the hyphae (apart from their distribution) indicated that they belonged to a fungus or fungi living in the wood, and did not result from accidental infection. To confirm this, other specimens, after treatment with running water, were externally sterilized by means of an alcoholic solution of corrosive sublimate, and were subsequently washed with sterilized water: yet the result was the same.

The white hyphae subsequently often became golden or tawny if they dried and remained sterile. Otherwise numerous green conidiophores soon arose, and resembled those of *Penicillium*. Later the conidiophores sometimes assumed a warm-bronze tint.

Artificial production of 'brown oak'.

In order to test the effect of the fungus on normal heart-wood, small cubical blocks of the latter were piled on top of one another in wide test-tubes, sterilized by steam, and infected by conidia of the fungus. The test-tubes were

kept upright, and in some cases the plugs of cotton-wool were covered with caps of tin-foil. At the bottom of the tube was previously sterilized water containing spores that had not adhered to any of the blocks. This arrangement ensured to the different blocks different degrees of moisture, the supply of moisture increasing from above downwards, the lowest block being permanently partly immersed in water.

The blocks in the middle of each column in the tube had the medium amount of moisture, and it was they that assumed a brown colour similar to that of 'brown oak' (in fact very similar to Messrs. Oliver's specimen), but varying towards that of fumed oak. The blocks partly immersed in water, and those at the top of the column in tubes, when no tin-foil caps were used, showed little or no change in tint. The *brown colour was assumed* therefore *only when the heart-wood contained moisture exceeding a certain minimum, and falling short of a certain maximum.* Such is one of the conditions of development in wood of all wood-destroying fungi.

In a second series of cultures in which larger boards of heart-wood were used, and the precautions against the intrusion of foreign organisms were less rigid, the boards showed the successive characteristic changes of tint from yellow to brown, including small patches of the rich brown of genuine 'brown oak'.

Distribution of the mycelium in the wood of the tree.

The colour of the hyphae emerging in culture from browning oak, the definite localization of these emerging hyphae, and the artificial production from heart-wood of wood simulating 'brown oak', all point to a causal connexion between fungus and browning process. This view is strengthened by the distribution and nature of the mycelium in the wood of the standing tree. Hyphae are absent from the sap-wood (and tissue outside it), and from parts of the normal heart-wood distant from the brown wood; they occur in an active living condition in regions of the heart-wood where conversion into brown wood is taking place, and, lastly, are present, mainly at least, in a dead and disguised form in 'brown oak' that has attained its final yet firm condition. The fact that in the mature brown oak the mycelium is wholly, or possibly nearly wholly, dead causes one to doubt if the decay observed in 'brown oak' is due to the same fungus; and additional reasons for this doubt will be given later in this paper.

STRUCTURE AND DEVELOPMENT OF 'BROWN OAK'.

(*a*) Sap-wood.

The structure and contents of the sap-wood are normal; starch was particularly abundant in the parenchyma, thyloses, and medullary rays.

In the sap-wood, normal and brown heart-wood, many fibro-tracheides

possessed a thick internal layer of the wall that assumed a faint lilac tint with iodine, a blue colour with iodized chloride of zinc, and refused to answer tests for lignified walls. The occurrence of this wall-layer in the sap-wood proves that in the 'brown oak' its lack of lignification is not due to fungal attack.

(*b*) Normal heart-wood.

The heart-wood near the sap-wood differed from this by the almost complete lack of starch (remnants of which occurred in isolated ray-cells and in thyloses of the large vessels), and by the abundance of tannin not only in all kinds of parenchyma, but also in the walls of the constituents, especially the fibro-tracheides, thyloses, and to a less extent ray-parenchyma.

(*c*) Brown heart-wood.

The 'brown oak' showed the same general distribution of tannin as in the normal heart-wood. But it also contained not only solid, including brown, substances in various constituents of the wood, but also hyphae.

For the purpose of cutting sections, the wood was softened by treatment with 50 per cent. commercial hydrofluoric acid (after being boiled and cooled repeatedly in water) and the wood blocks were eventually kept in a solution of glycerine, alcohol, and water. The sections were further treated with water if mounted in glycerine jelly, and, if mounted in Canada balsam, were for a time immersed in absolute alcohol and xylol.

After such drastic treatment normal heart-wood had almost or entirely lost its tannin, and showed no solid nor coloured contents. 'Brown oak', on the contrary, even in its incipient stage still showed tannin in its walls and in certain solid brown bodies that occurred in various structural constituents. These brown bodies agree in reactions with the so-called 'wood-gum' or 'wound-gum' of wood.

1. The substance is insoluble, and does not swell appreciably in water, alcohol, xylol, concentrated hydrochloric acid (12 hours), concentrated sulphuric acid (12 hours), equal parts of concentrated ammonia and caustic potash solution (12 hours), nor successively in the last named and strong hydrochloric acid. (In the case of brown oak that had reached its final condition and was treated with concentrated sulphuric acid, there seemed to emerge from the brown bodies in question bubble-like drops of a colourless substance. These drops were probably derived from fungal hyphae concealed within the brown substance.)

2. With phloroglucin and hydrochloric acid the brown substance sometimes assumed a carmine colour and thus agreed with typical wood-gum, but even in such cases it refused to respond to Mäule's test for lignification; in other cases it remained yellow with phloroglucin and hydrochloric acid. Possibly the substance is never lignified, and owes its occasional response to

the phloroglucin test to the presence of one of the number of substances capable of causing the carmine coloration.

3. The substance is singly refractive.

As the chemical nature of such wood-gum (in wound-wood, true heart-wood, and false heart-wood) is unknown, and as it is not wood and there is no evidence that it is gum, in the sequel the substance in question will be termed 'the brown substance'.

Tannin is present in the brown bodies under discussion. To its presence they owe their blue-black coloration in ferrous sulphate, and deep staining in methylene blue, lactic blue; also their deepened and lightened tint in caustic potash and hydrochloric acid respectively. But tannin is not an essential part of the substance in question, as parts of one and the same brown body are respectively devoid and possessed of tannin.

As the term 'tannin' is here used merely to indicate a substance that responds to the test for certain tannin-bodies by assuming (in this case) a blue or blue-black colour with ferrous sulphate, it follows that the tannin present in the brown heart-wood is either different from that of normal heart, or if identical is in larger quantity, or is held more firmly by the walls (and brown substance). The second possibility is excluded by the analysis given later in this paper.

Biology and Effects of the Fungus.

For the sake of clearness, the succeeding description refers to wood that had been subjected to the treatment (boiling, hydrofluoric acid, and so forth) which has been described, and which had removed the tannin more or less completely from cells not changing nor changed into 'brown oak'.

(*a*) **Heart-wood of normal colour.**

Immediately within the normal heart-wood (devoid of hyphae) was a region of the same colour and for the most part free from hyphae, yet here and there showing some of these in vessels and wood-parenchyma. These hyphae were often dotted with glistening globules, but no masses of 'brown substance' occurred. Nearer to the more central 'brown oak' the main mass of the wood was devoid of hyphae and brown substance, though both of these showed (in tangential sections) sporadic patches of cells in the medullary rays containing hyphae and the 'brown substance', *which was yellow.*

Still nearer to the 'brown oak' the heart-wood, either normal or more yellow in colour, showed hyphae and brown substance arranged in longitudinal strands, and in some medullary rays, while the remaining tissue was normal in contents. This distribution of hyphae and brown substance in longitudinal strands thus is preparatory to the later stage already described, in which the wood is traversed by brown stripes. Both are due to the fact

that the hyphae advance most rapidly in a longitudinal direction in the vessels and circumvasal tissue, which form radial series. In a transverse plane advance of the hyphae is most rapid in a radial direction by means of the medullary rays.

These facts, including the slow advance of the hyphae in a tangential direction, help to explain the cases where the 'brown oak' advances and tapers from the base of the trunk upwards, or where it becomes restricted to one side or one of two stems in a double-stemmed oak, though in the first case the taper of the heart-wood itself may intervene. Further, the mode of advance of the fungus (coupled with the very feeble power that the fungus has of attacking lignified walls) at least partially accounts for the obstructive influence of large knots.

(*b*) **Brown heart-wood mainly in the form of longitudinal strands.**

In this stage and the preceding one in which the hyphae and brown substance are in strands, though not clearly marked to the naked eye, all steps in the advance of the hyphae and in the manufacture of the brown substance are to be seen. Both stages will therefore be described together.

The *advance of the hyphae along the medullary rays* was revealed especially clearly in the normal-coloured heart-wood in places where hyphae and brown substance were abundant in the ray, but absent or scanty in the neighbouring vessels, tracheides and parenchyma. In the rays the hyphae run mainly in a transverse radial direction, passing through the copious pits of the terminal cell-walls. Yet here and there they emit branches to the tissue on their flanks; for often the wood-parenchyma in the immediate vicinity of infected ray cells also contained hyphae and brown substance, whereas wood-parenchyma tangentially more remote lacked these.

In uniseriate and multiseriate rays alike the cells containing the brown substance always entertained hyphae, which could be traced farther out in the ray towards the normal wood than could the brown substance. With this exception very few of the colourless ray-cells in the infected region contained hyphae. These facts show that *the brown substance is the effect*, not the cause, *of the presence of hyphae*. In the medullary rays of the Edinburgh specimen the hyphae extended outwards approximately to the same distance as the first granular traces of the brown substance.

In the vessels, tracheides, and wood-parenchyma the course of the hyphae is generally longitudinal. Consequently in transverse section there are isolated islands of vessels and surrounding tissue containing hyphae and brown substance. The actually contiguous uniseriate rays may be devoid of hyphae or contain these only where the ray actually crosses the infected island. Traced further outward such a ray is devoid of hyphae until another infected island is touched, when hyphae may reappear in it. Thus as they travel along the vessels and circumvasal tissue hyphae can infect

rays at successive levels. Such a mode of infection was particularly clear in the case of multiseriate rays, which crossed infected strands of vessels and showed hyphae and brown substance merely in their sides towards the infected vessels.

Occasional hyphae were seen running tangentially in tracheides to or from other tracheides and a medullary ray.

(*c*) Production of the brown substance.

The following stages were observed in the hydrofluoric-acid material:

1. Hyphae entering colourless parenchyma cells or vessels were studded with glistening globules. And in the Edinburgh material such was the case with parts of the hyphae freely traversing the lumen of the cell, and not in contact with the cell-wall.

2. Cells showed granular colourless, or very faintly yellow, contents in proximity to the active intracellular young hyphae.

3. The contents were definitely yellow, in larger quantity, and formed a homogeneous hyaline mass surrounding the hyphae.

4. In the final stage, as seen in completely 'brown oak', the substance is definitely brown, present in still greater quantity, and may fill the parenchyma-cell, except where it is permeated by dead, often almost unrecognizable, hyphae, which are separated from it by a clear space.

These stages indicate that the brown substance is deposited outside the hyphae as colourless globules, which later increase in number and undergo some change so as to give rise to a yellow mass of granules or globules; these in turn remain in or assume a colloid condition to form eventually a homogeneous solid mass of constantly deepening colour. The appearances presented suggest that the substance is excreted by the fungus, but an alternative suggestion is given in the sequel where the food of the fungus is discussed.

(*d*) An additional plugging substance.

Mention must be made of an entirely different solid substance of unknown nature present even in the material, subject to treatment with hydrofluoric acid and so forth. This substance, granular in nature, when seen in thin sections often showed a yellowish tinge, but when seen in thicker masses was dark and opaque. Its occurrence and distribution were independent of those of the hyphae. It occurred in normal heart-wood as abundantly as in brown heart-wood. In distribution it was localized, often being found in longitudinal strips, especially in wood-parenchyma and ordinary tracheides, but also in the adjoining thick-walled fibro-tracheides. In such cases it was present in the uniseriate rays traversing the strip solely where the former crossed the latter.

(*e*) Action of the fungus on lignified walls.

The effect of the fungus on the mechanical strength of the heart-wood is so small that 'brown oak' is an excellent material for panelling and furniture. This corresponds to the fact that the fungus attacks lignified walls as visible structures feebly and slowly. It appears to pass from one cell or vessel to another solely through the pits. This was particularly evident in the case of hyphae traversing the terminal walls of wood-parenchyma and ray-parenchyma; and cases were observed in which a hypha on reaching a spot on the wall where no pit occurred executed a bend and so reached the nearest pit.

The constituents of the wood for the most part retain not merely their visible structural integrity but also their lignified condition. Yet the fungus has some power of delignifying wood. Here and there where two vessels, or a vessel and a tracheide, or two tracheides, were in lateral contact, the half of the wall belonging to the constituent containing hyphae gave a cellulose reaction in the vicinity of these, but a lignified reaction on the side towards the constituent devoid of fungi. In a more advanced stage of attack the wall was locally delignified throughout its thickness. This restricted power of delignification appears to begin at the pits, for the section of a wall separating two tracheae sometimes showed alternate minute patches of cellulose and lignified substance, each patch embracing the whole thickness of the wall. Each cellulose patch probably corresponded to a pit whose plane lay outside the section. Occasionally thin sections showed real gaps in the walls separating two vessels, although I never succeeded in proving beyond doubt that these were due to the fungus, and not to the razor. Probably owing to its weak power of attacking lignified walls, and its exclusive or nearly exclusive passage through pits, the fungus occurs very scantily in fibro-tracheides.

(*f*) Source of nutriment of the fungus.

As the fungus is confined to the heart-wood, and makes so slight an attack on the visible structure of the lignified walls, the question arises as to the source of its food. Available are: in the lignified walls, pentosans (xylan and so forth), pectic bodies, glucosides, and tannin, as well as the cellulose and substances causing the 'lignin' reactions. Tannin is also available in lumina of the parenchyma, including that of medullary rays, and thyloses. Whatever be the precise food substances utilized, the *method of nutrition of this fungus is novel*, so far as our present knowledge is concerned, though the future will probably reveal other wood-inhabiting fungi of similar feeding habits, possibly associated with the inception of firm wound-wood or firm false heart-wood.[1]

[1] See E. Münch, 'Über krankhafte Kernbildung'. Naturwiss. Zeitsch. f. Forst- und Landwirtschaft, 1910, Heft 11.

Several sets of facts favour the view that *tannin is used as food-material.*

1. An analysis of 'brown oak' compared with the normal heart-wood showed that the latter contained 13·33 and the former 10·05 per cent. of tannin. This would represent a loss of nearly 25 per cent. of the original tannin. But in the absence of analyses of oak timber at different depths inwards in ordinary heart-wood, it is conceivable that the smaller amount in the brown oak is not due to the fungus nor associated with the 'browning'.

2. The distribution of the fungus and tannin are of significance in this connexion. (For purposes of observation the wood was softened by dilute glycerine, induced to enter by means of an exhausting air-pump: no heat was applied.)

In the fully brown heart-wood tannin was generally diffused in the lignified walls, but especially in the lumina of the wood-parenchyma, thyloses, and cells of the medullary rays. In all uniseriate and multiseriate rays there was tannin, which often was most abundant in the marginal cells and lacking from many other cells. It was in all three forms of parenchyma that the hyphae and brown substance were also most abundant. In one and the same cell frequently parts of the brown substance contained tannin, which was absent from other parts of the same mass in the same cell.

In regions where conversion of the heart-wood into 'brown-oak' was taking place some of the cells of the wood-parenchyma and medullary rays were poor in tannin, or devoid of it, and at the same time free from hyphae; whereas contiguous cells in the same tangential or radial series contained hyphae and richer stores of tannin. Again, in some cells there was no tannin except a thin film in (or on?) the hyphal wall, or isolated minute droplets studding the hypha, which sometimes also traversed a larger globule of tannin: in these cases the tannin stained unusually light blue (a somewhat dark cobalt-blue) with ferrous sulphate, yet was sufficiently concentrated to stain deeply with lactic blue.

These facts are all capable of two opposed interpretations, namely that the hyphae consume or excrete tannin. The view more consistent with evidence derived from other sources is that the hyphae preferentially enter tannin-containing cells, and consume the tannin until in the absence of fresh supplies tannin is so reduced in quantity as to be a dilute solution in the form of a film or minute globules on the hypha. After that stage the tannin may be wholly absorbed. But in addition to the tannin present in the cells, there is that in the walls; this may be liberated from the wall by the solution of some ingredients in the wall or by water or a solution excreted by the fungus, and may be deposited in the accumulating brown substance.

This view that the fungus feeds at the expense of the tannin is strengthened by *cultures* made of the fungus *in solutions of tannin.* Conidia of the fungus were sown in 0·05, 0·25, 0·5 per cent. solutions of commercial

tannin (which is not identical with the tannin of oak wood). When bacteria were excluded vigorous submerged mycelia developed, but the solutions did not darken in tint. When the mycelia were accompanied by bacteria, derived from cultures from the original wood, the solutions darkened. Inside 'brown oak' I failed to find any bacteria.

IDENTITY OF THE FUNGUS.

Repeated trials as to the source of the *Penicillium*-like conidiophores emerging from incipient 'brown oak' showed that these belonged to the fungus causing the process of browning. This was confirmed not only by the physiological action of the mycelia in cultures made in oak heart-wood, but also by the occurrence of minute *Penicillium*-like conidiophores in the narrow vessels of the intact 'brown oak'.

As regards other stages of the life-history of the fungus, I did not succeed in obtaining by means of cultures derived from conidial infections any other stage. But from the regions where the hyphae were still active on samples of the 'brown oak' from England and Scotland there were produced little spheroidal brownish-yellow fructifications, whose diameter did not exceed that of half a pea-seed. These appeared to belong to the Plectobasidiinae. Being unable to identify these, I submitted them to Mr. George Massee, who states that they are the basidiocarps of *Melanogaster variegatus*, Tul., var. *broomianus*, Berk.

SUMMARY.

In certain individual British oak-trees (*Quercus Robur* [1]) the ordinary heart-wood is partially replaced by a rich-toned, often reddish, brown wood, which is firm and hard, and is termed 'brown oak'.

Under the influence of a septate fungus living exclusively in the heart-wood normal heart-wood is changed in 'brown oak'. The fungus therefore presumably infects solely, through a wound, trees sufficiently old to possess heart-wood.

'Brown oak' usually occurs at the base of the trunk and the adjoining root, and generally tapers upwards in the stem and downwards in the root. But the fungus can gain entrance to upper parts of the tree and so produce in these regions masses of 'brown oak', even in individuals devoid of it in their lower parts.

The fungus in the infected tissue is responsible for the production of a brown substance (or brown substances) highly resistant to solvents and responding to the reactions of the ill-defined material termed 'wound-gum' or 'wood-gum'. This arises in the form of colourless or faintly yellow globules or granules, which eventually aggregate to form brown masses in

[1] This name is used in its main historic sense as including *Q. pedunculata* and *Q. sessiliflora*. From which of these species my material was derived, and the extent to which 'brown oak' occurs in the two, are unknown to me.

the cavities of the wood-constituents. The change of tint of the heart-wood as a whole and the production of the brown substance in the individual cells definitely succeed the entry of the hyphae. Artificial infections of fragments of normal heart-wood caused this to assume colours approximating to or agreeing with those of true 'brown oak'.

The fungus (and colour change) advances most rapidly in a longitudinal direction along the lines of vessels and circumvasal tissue, and in a transverse direction along the medullary rays: the advance in a tangential direction is comparatively slow. These facts find at least partial explanation, first, in the extremely limited power of the fungus to attack and delignify lignified walls, and in the consequent advance from constituent to constituent exclusively through pits or pores; secondly, in the circumstance that the fungus thrives particularly in parenchyma (wood and ray) in which it runs mainly in the direction of the long axes of the cells, passing out through the numerous pits in the terminal walls.

Among the consequences of the mode of advance and limited power of dealing with lignified walls are the following:

(*a*) 'Brown oak' can remain firm and hard in the tree for a long time. And since the mycelium of the fungus concerned in mature 'brown oak' is largely, if not entirely, dead (even though conidia may occur in it) there is no reason to believe that the obvious decay of 'brown oak' occurring in some cases is due to this fungus. Such decay may be induced by other wood-destroying fungi that attack normal heart-wood of the oak.

(*b*) In early stages of conversion of heart-wood into 'brown oak' the latter is seen in its incipient condition as longitudinal darker bands traversing normal coloured wood. This condition is reflected in and explains the tortoiseshell variety of mature 'brown oak'.

(*c*) The advance of the process of browning is arrested or obstructed by large knots, though burr-wood with numerous small knots may be completely brown.

(*d*) Associated, at least partly, with the limited power possessed by the hyphae of advancing in a transverse and above all in a tangential direction, are the cases where brown oak becomes limited to one side of a stem, or to one or two among several 'leaders' into which the infected trunk divides.

The source of the food of the fungus constitutes a complex chemical problem at present insoluble by microchemical methods. That tannin is one of the sources is suggested, first, by the development of the fungus particularly in tannin-containing constituents of the heart-wood; secondly, by the power of the fungus to obtain all its essential organic food from commercial tannin; and, thirdly, by the smaller quantity of tannin in 'brown oak' than in the adjoining normal heart-wood (of the one specimen investigated).

The fungus responsible has conidiophores closely resembling those of

Penicillium. On incipient 'brown oak' of Oliver's and Borthwick's specimens there eventually were produced small spheroidal basidiocarps which Mr. George Massee identifies as *Melanogaster variegatus* var. *broomianus*. The identity of the conidiate fungus with that of the basidiate one was not established by pure cultures. If, however, the wholly or partially subterranean species of *Melanogaster* in question is the fungus responsible, infection by hyphae or spores through a wound in or near the root would appear to be especially simple, and might be partly responsible for the preponderance of 'brown oak' near the base of the tree. In this connexion may be mentioned the fact that the main root of 'brown-oak' trees is often found to be destroyed.

That the production of 'brown oak' is not due to the direct action of a particular chemical ingredient of the soil is proved by the distribution of this wood in the individual tree as well as by the occurrence side by side of normal and 'brown-oak' trees.

In conclusion I express my thanks to Professors H. Brereton Baker, F.R.S., and J. F. Thorpe, F.R.S., for securing quantitative estimations of the iron and tannin respectively, and to Mr. W. P. Rial for performing the analysis in the case of the latter; to Messrs. Alexander Howard and Stuart Oliver for valuable information; to Messrs. Borthwick, Bradley, and E. T. and S. Oliver for kindly supplying fresh specimens; and to Mr. George Massee for his identification of the fungus.

APPENDIX.

By Mr. W. P. Rial.

Tannin in Oak heart-wood.

The wood was taken in the form of fine shavings across the grain: 9 grammes of this were placed in an extracting apparatus and extracted for 1 hour with 225 c.c. water. The residue was then extracted for another hour with 225 c.c. water, and at the end of this time a few drops of the liquid which was passing through the wood were tested with $FeCl_3$ and found to contain no tannin. The extract was placed in a 500 c.c. measuring flask and made up to 500 c.c.

This was done for both specimens in a similar manner.

The tannin present was estimated by the method used at the Yorkshire College.

25 c.c. of an indigo carmine solution are added to 750 c.c. H_2O and $KMnO_4$ added until a pure yellow colour is obtained.

The titration is then carried out in presence of 5 c.c. of tannin extract, and also in presence of 20 c.c. of pure tannin solution.

RESULTS.

25 c.c. indigo solution required $\left.\begin{matrix}43{\cdot}9\\44{\cdot}1\end{matrix}\right\}$ 44·0 c.c. $KMnO_4$

" + 5 c.c. extract ordinary oak 67·5 c.c. "

" + " " brown " 61·7 " "

" + 20 c.c. pure tannin solution 61·5 " "

The pure tannin solution was made by dissolving 0·1118 grm. of pure tannin in water and making up to 250 c.c.

20 c.c. pure tannin solution $\rightarrow 61{\cdot}5 - 44{\cdot}0 = 17{\cdot}5$ c.c. $KMnO_4$

i.e. 1 c.c. $KMnO_4 \rightarrow \frac{20}{17{\cdot}5} \times \frac{{\cdot}1118}{250}$ grm. tannin.

$= 0{\cdot}00051$ grm. tannin.

5 c.c. ordinary oak extract $\rightarrow 67{\cdot}5 - 44 = 23{\cdot}5$ c.c. $KMnO_4$

$23{\cdot}5$ c.c. $KMnO_4 \rightarrow 23{\cdot}5 \times 0{\cdot}00051$ grm. tannin.

∴ 500 c.c. " " $23{\cdot}5 \times 100 \times 0{\cdot}00051$ grm. tannin, and this is contained in 9 grm. of wood.

$$\therefore \% \text{ tannin} = \frac{23{\cdot}5 \times 100 \times 0{\cdot}00051}{9} \times 100$$

$$= 13{\cdot}33.$$

5 c.c. brown oak extract $\rightarrow 61{\cdot}7 - 44 = 17{\cdot}7$ c.c. $KMnO_4$

$17{\cdot}7$ c.c. $KMnO_4 \rightarrow 17{\cdot}7 \times 0{\cdot}00051$ grm. tannin.

∴ 500 c.c. contain $100 \times 17{\cdot}7 \times 0{\cdot}00051$ grm. tannin, and this is contained in 9 grm. wood.

$$\therefore \% \text{ tannin} = \frac{17{\cdot}7 \times 100 \times 0{\cdot}00051}{9} \times 100$$

$$= 10{\cdot}05.$$